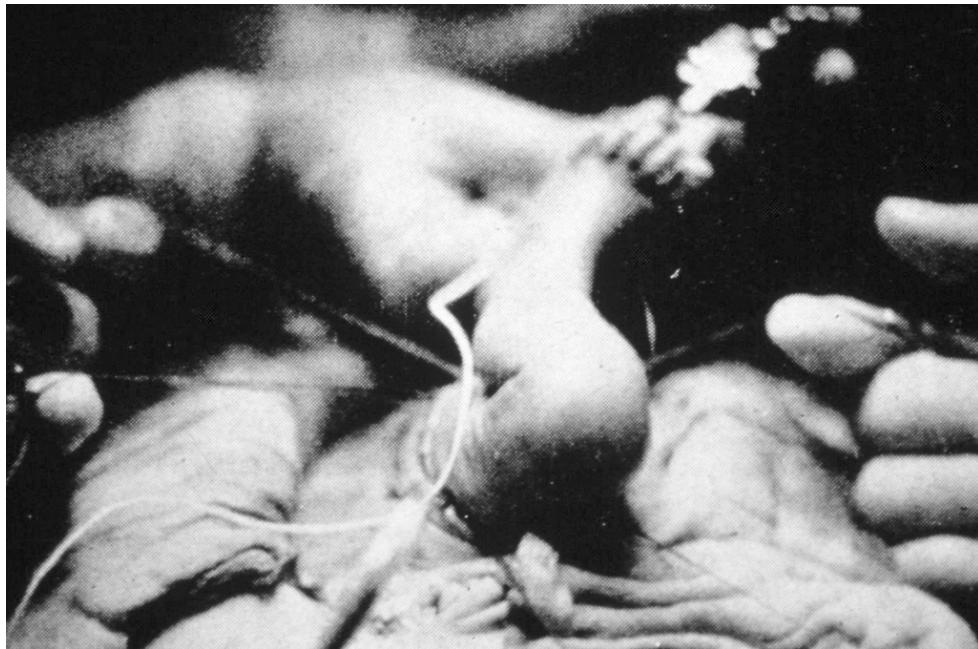


Fetal Blood Group Genotyping

Greg Denomme, PhD
Canadian Blood Services and
Mount Sinai Hospital

Today's Objectives

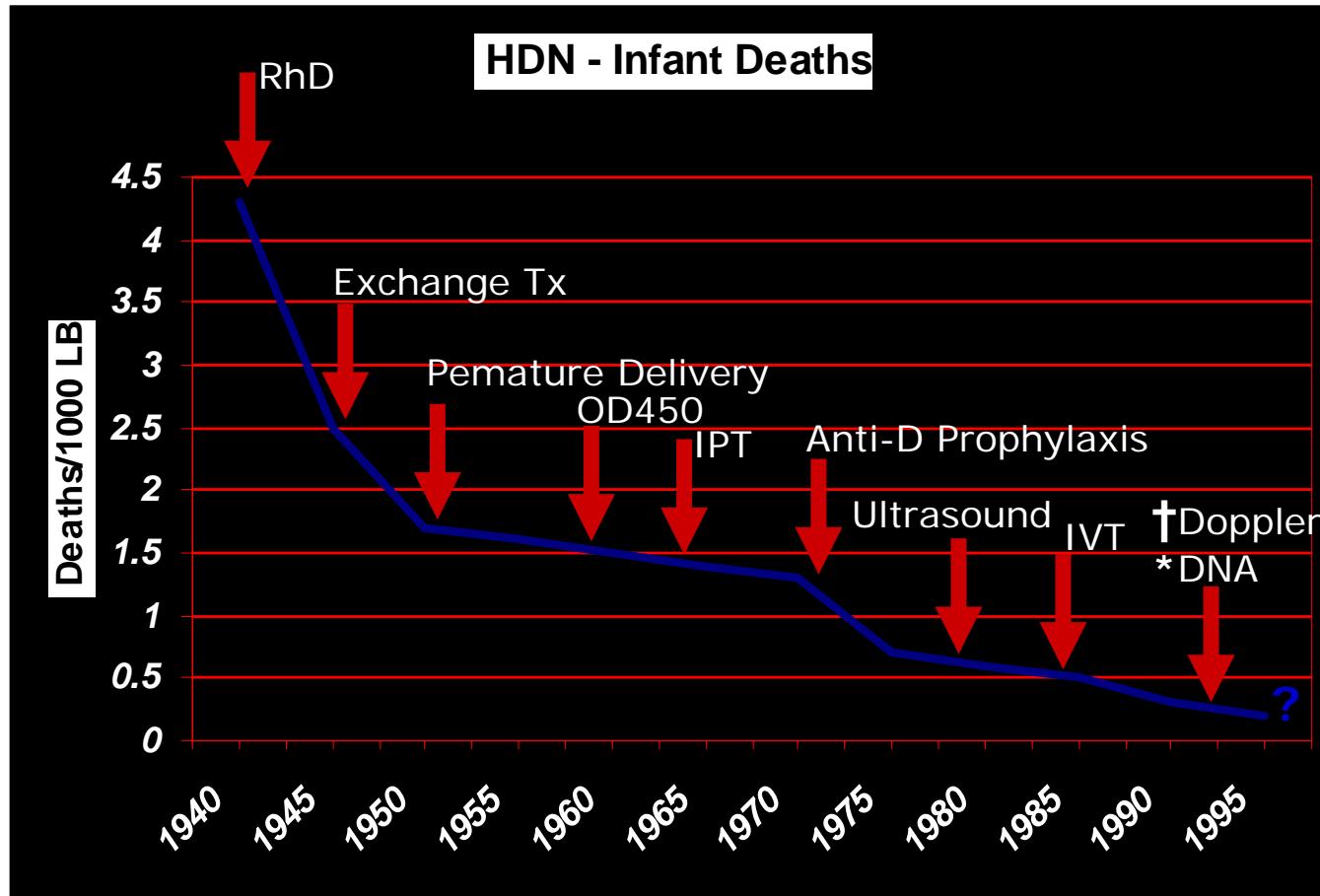
- Historical perspective
 - Why do predictive genetics?
- Predictive Genetics
 - Paternal zygosity
 - Fetal DNA (geno)typing
- Provide impact data
 - Are we making a difference?
- Review state-of-the-art management for this disorder
 - Fetal DNA in maternal plasma



Freda VJ. Exchange transfusion *in utero*. *AJOG* 1964;89:817-21

Predictive Genetics in HDFN

Anti-D HDFN



*Bennett PR, et al. *N Engl J Med* 1993;329:607-10

*Wolter LC, et al. *Blood* 1993;82:1682-3

†Oepkes D. *Obstet Gynecol* 1993;82:237-41

Sources of Fetal DNA

- Chorionic villi – limb deformities (amnio bands)

Scott R. *Lancet* 1991;337:1038-9

- Cervical plug – variable, not reliable

Griffith-Jones, MD. *BJOG* 1992;99:508-11

- Amniotic fluid: (≤ 20 weeks)

- amniocytes (1° culture) mostly dead epithelial cells

Fauza D. *Best Pract Res Clin Obstet Gynaecol* 2004;18:877-91

- Fetal DNA in maternal plasma

Lo YM. *Lancet* 1997;350:485-7

Procedural Risks

Procedure	TPH	↑Ab Titre	Death
• Cordocentesis	33%	25%	3-10%
	Wilson RD. <i>Fetal Diagn Ther</i> 1994;9:142-8		
• Amniocentesis • (17-20 weeks gestation)	2%	3%	0.3%
	Aickin DR. <i>J Ob Gynaecol Br Commonw</i> 1971;78:149-54		
• Maternal blood • (Fetal DNA in maternal plasma)	0%	0%	0%

Genotyping Strategy (1993)

Biological father is heterozygous:

- History: moderate to severe HDFN
- Antibody titre: moderate to high
- Amniocentesis (prefer ≤ 20 wks often 24+ wks)
 - Fluid \rightarrow OD450nm bilirubin estimation
 - Cells gDNA \rightarrow fetal blood group (geno)type
- \rightarrow Antigen neg: return to primary care
- \rightarrow Antigen pos: tertiary care management

45% of pregnancies are antigen compatible

Paternal *RHD* Zygosity

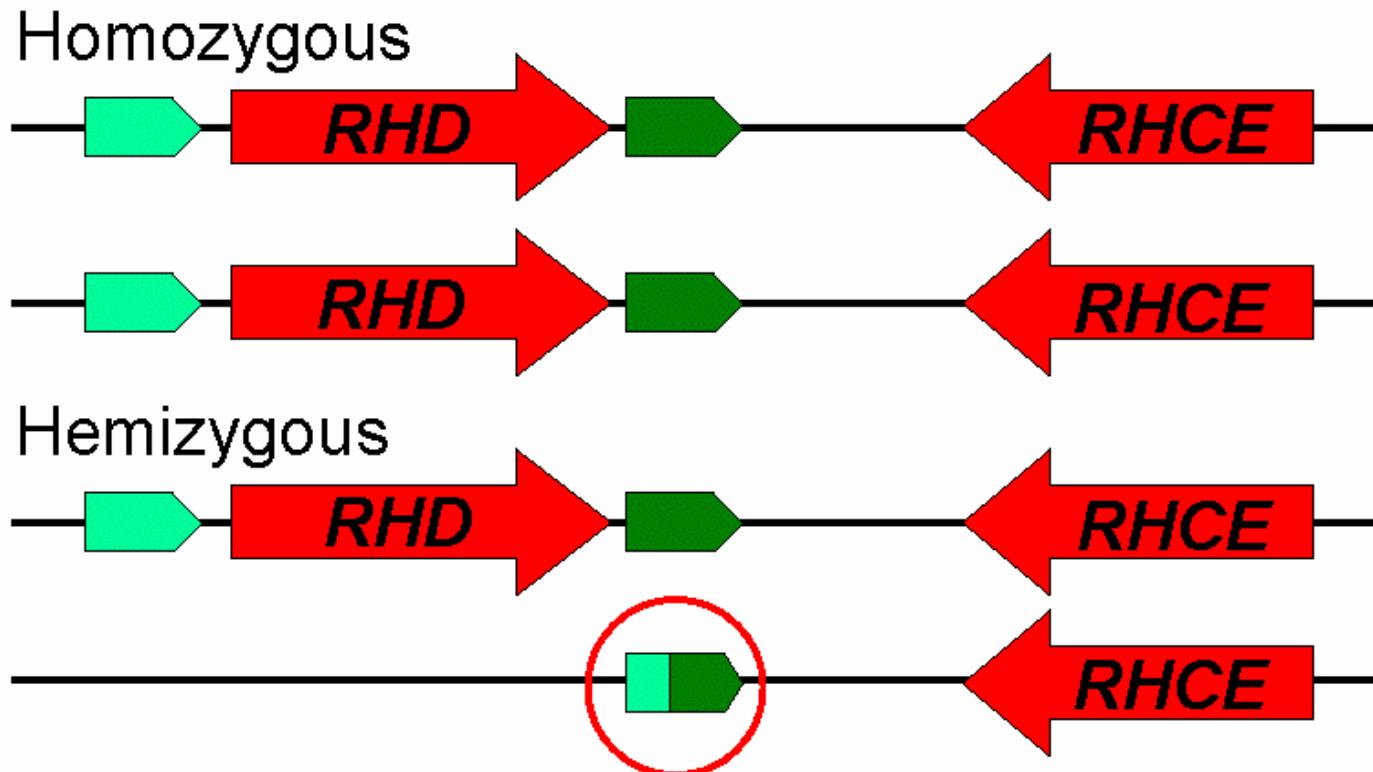
Homozygous vs Hemizygous
Copy number of functional genes

Most probably genotype

Rh Phenotype	Possible Genotypes	Pop'n Frequency (%) Caucasian W. African
D+C+E+c+e+	R_1R_2	12
	R_1r''	1
D+C-E-c+e+	R_oR_o	25
	R_or	25

Mourant AE. The distribution of human blood groups and other polymorphisms. 2nd Ed. London, Oxford University Press, 1976.

Rhesus box analysis

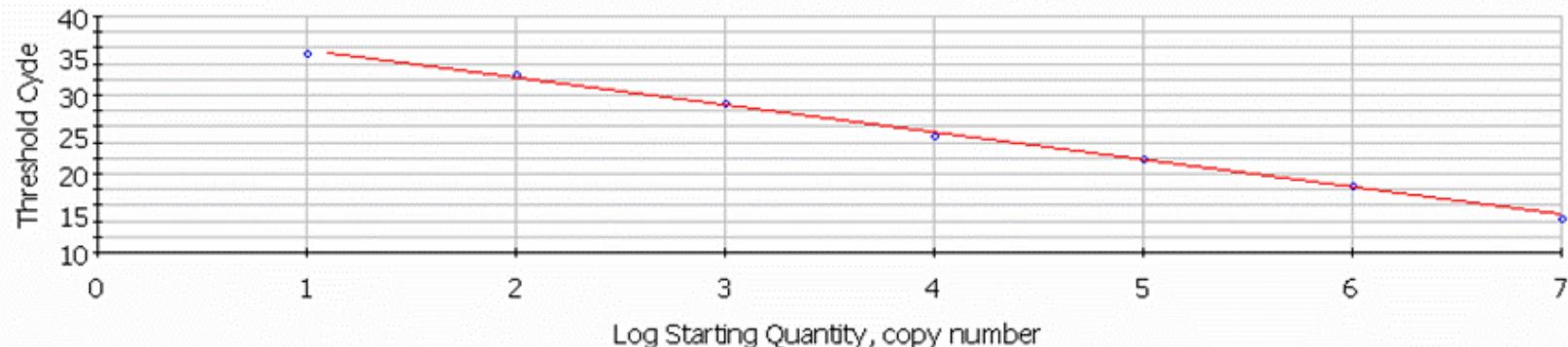


Wagner FF, Flegel WA. *Blood* 2000;95:3662-8

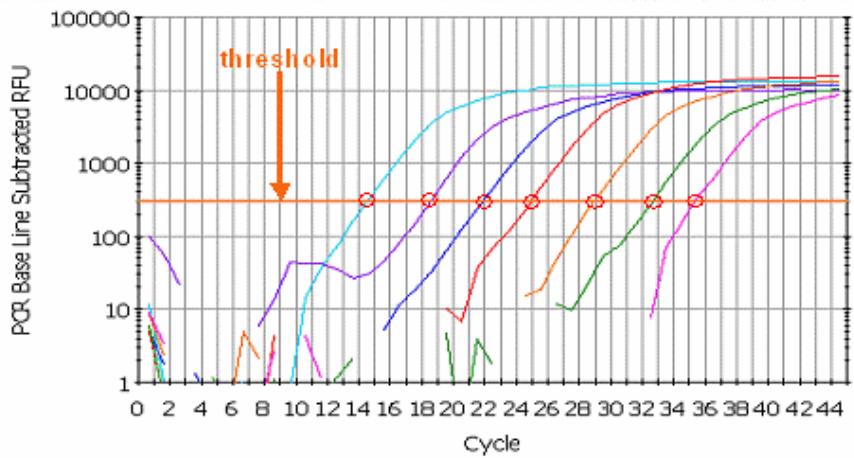
Quantitative PCR

Correlation Coefficient: 0.999 Slope: -3.488 Intercept: 39.204 $Y = -3.488 X + 39.204$

■ Unknowns
○ Standards



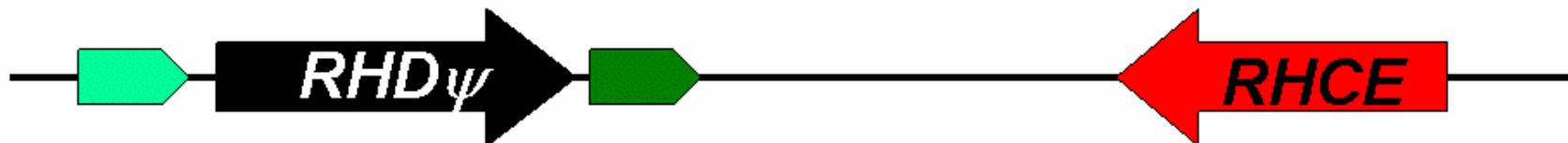
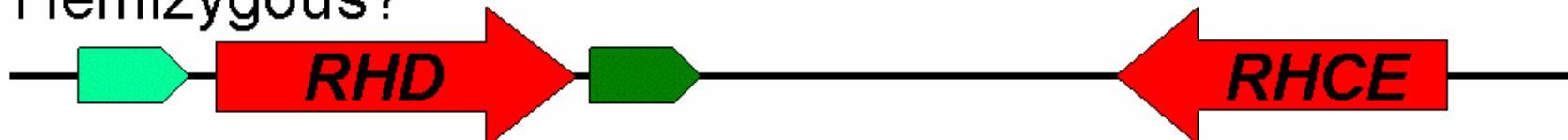
PCR Standard Curve: Data 27-Jan-03 1233ileff.opd



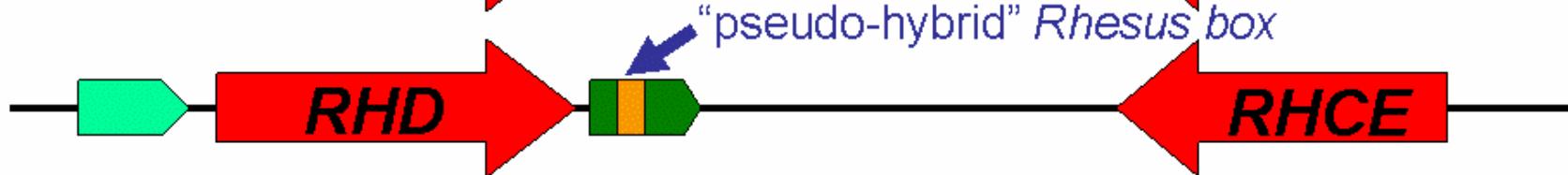
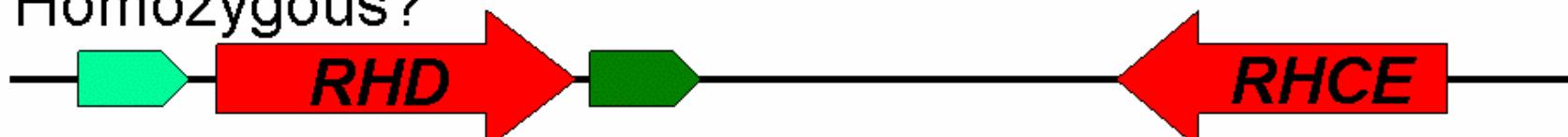
Chiu RW. *Clin Chem* 2001;47:667-72

Confounders

Hemizygous?



Homozygous?



Denomme GA. *Transfusion* 2002;42:645-50

Shao C-P. *Transfusion* 2003;43:335-9

Grootkerk-Tax MG. *Transfusion* 2005;45:327-37

Wagner FF. *Transfusion* 2005;45:338-44

Kim JY. *Transfusion* 2005;45:345-52

Future of *Rhesus box* analysis

- Novel upstream and downstream *Rhesus boxes* confound PCR-RFLP and double-amplification refractory mutation analyses
- Non-functional *RHD* alleles confound gene dosage analysis
- Compromises:
 - Two assays with combined high specificity (?)
 - Long range high-fidelity PCR to identify the *RHD*-negative haplotype

Fetal Blood Group Genotyping

Clinically Relevant Antigens

- D, E, c, e, C (if D neg)
- K, Kp, S, (s), M, (N)
- Fy, Jk
- HPA-1, 3, 5
- Others <1%

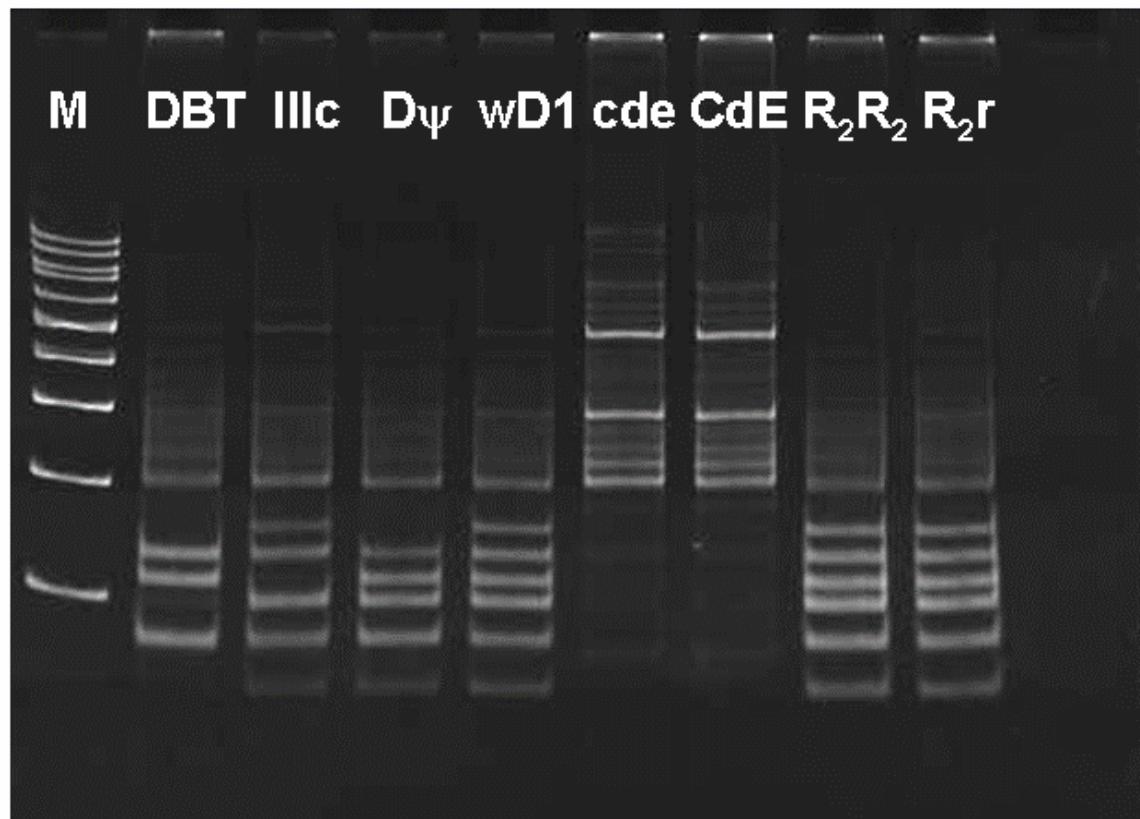
Techniques:

PCR-RFLP (preferred)

SSP-PCR (C, M, S)

Sequencing (Mil)

Fetal *RHD* 'typing'



Mother (obligatory):

RHD multiplex
[*RHD* ψ , *Cde*^s]

Fetus:

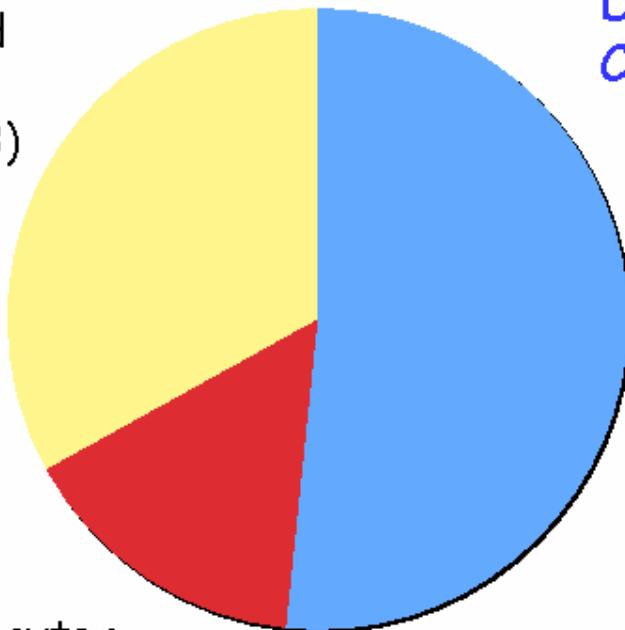
RHD multiplex
RHD ψ
[Repeat cAmino]

Maaskant-van Wijk PA. *Transfusion* 1998;38:1015-21.
Erratum in: *Transfusion* 1999;39:546

Samples Submitted for Analysis

AF & cultured
amniocytes
33% ('97 - '03)

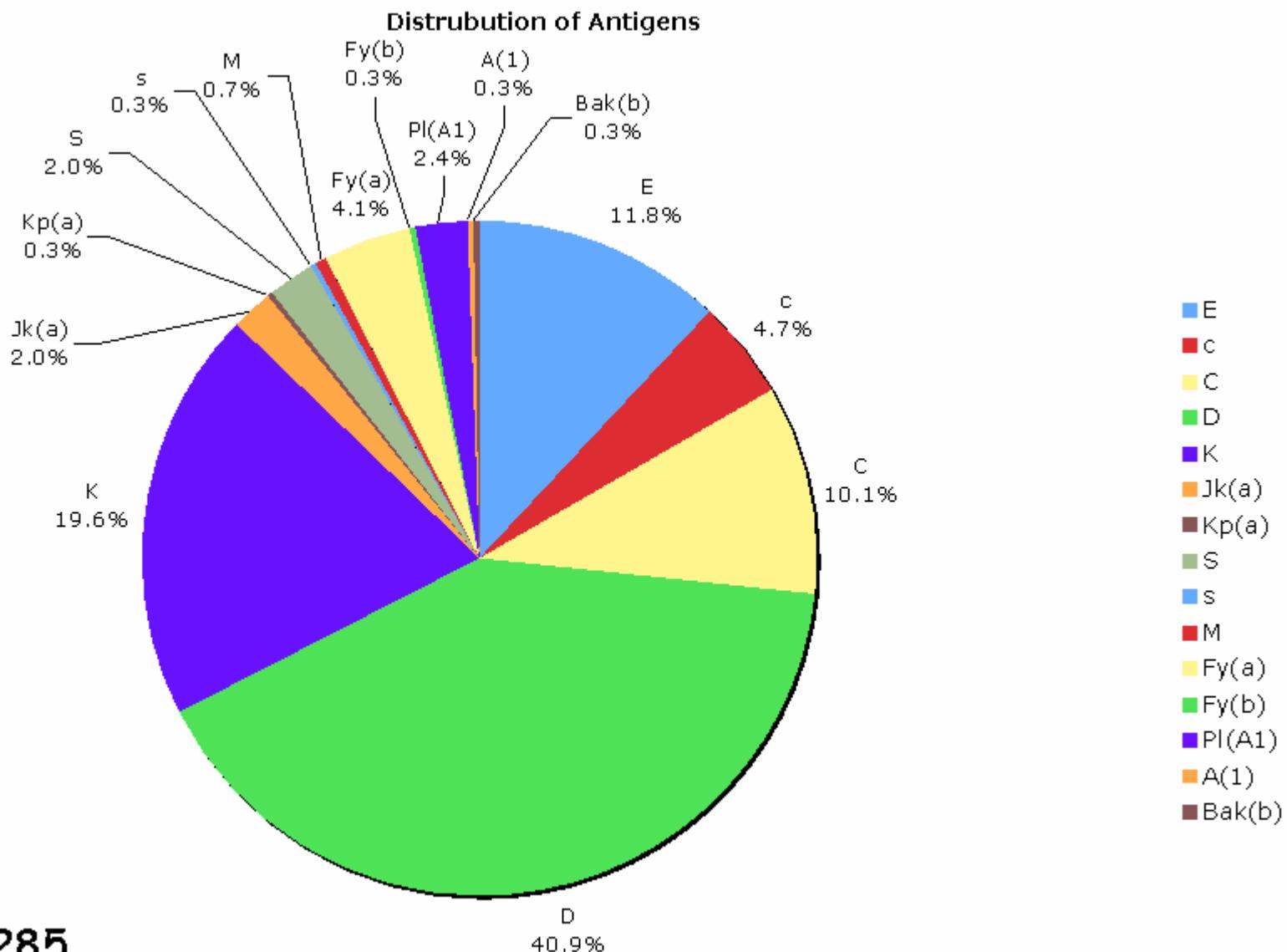
Cultured amniocytes
15%



10 mL amniotic fluid:
DNA extraction (8 mL)
Cultured amniocytes (2 mL)
- use when inconclusive

Direct amniotic fluid
52%

T25 flask of cultured amniocytes

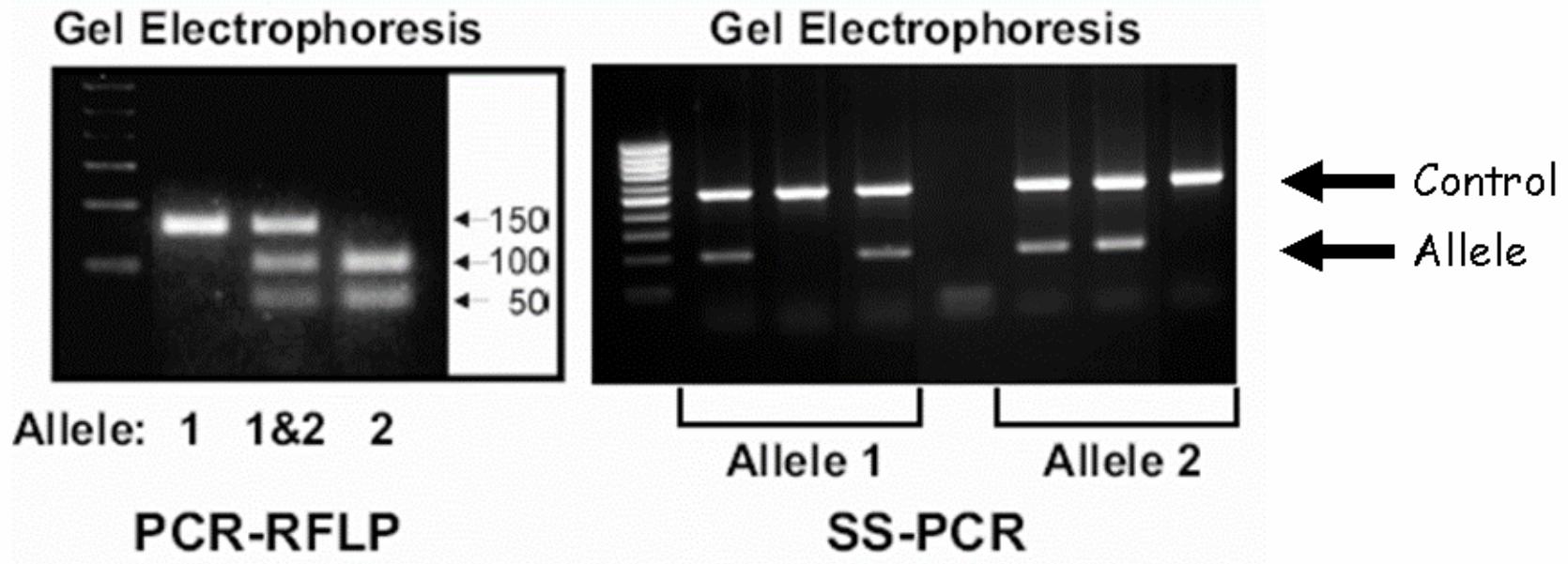


N = 285

PCR-RFLP vs SSP-PCR

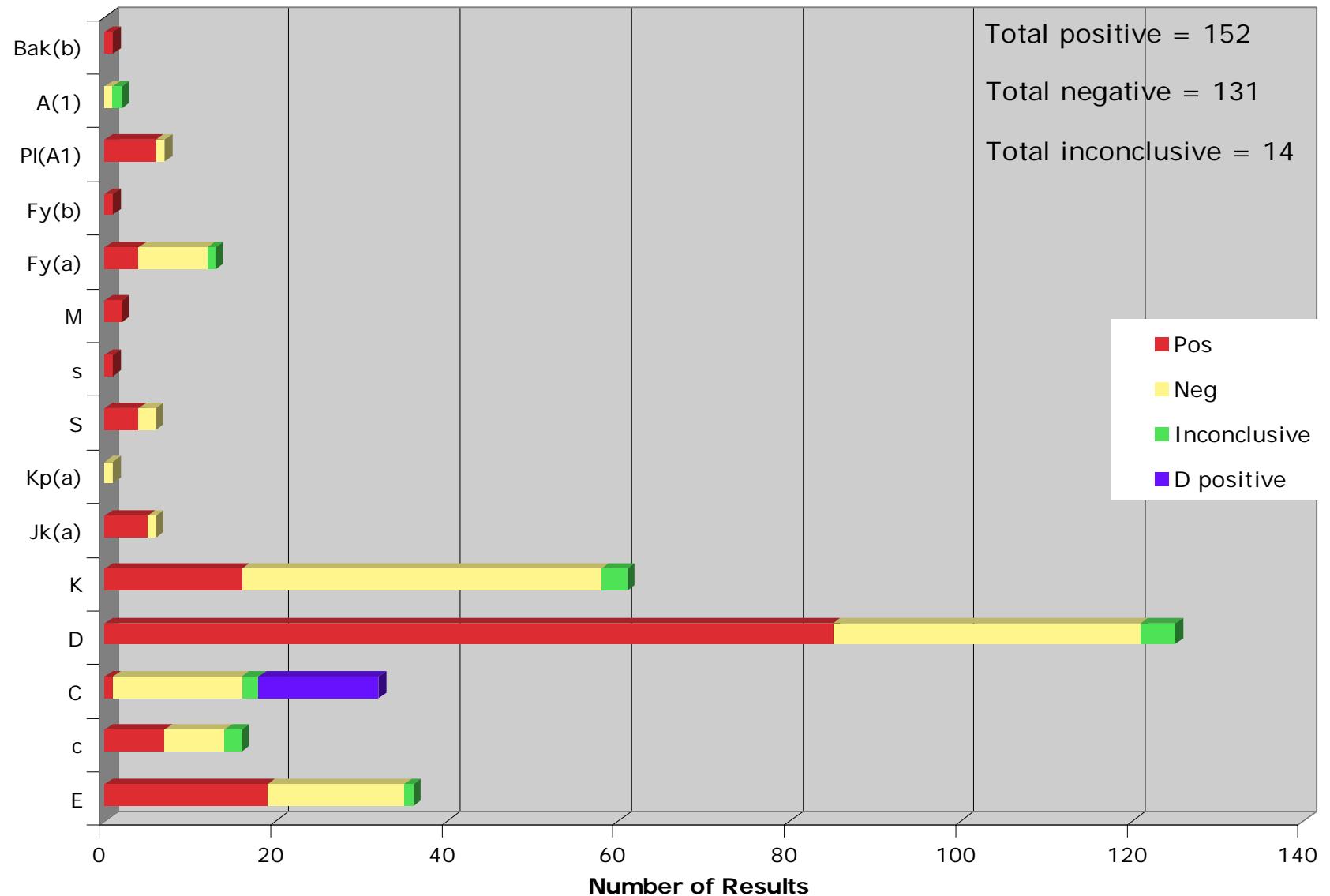
Lessons from *K1* SSP-PCR (June 1997 to Oct 1998)

2 of 14 AF results false negative when
repeated using cultured amniocytes



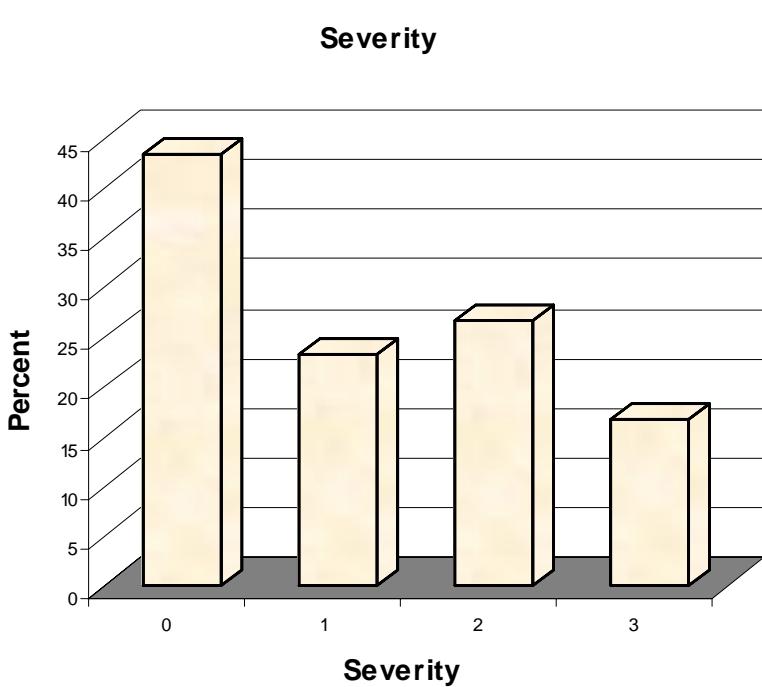
Allele PCR bp size must be less than the control!

Distrubution of Antigenic Results

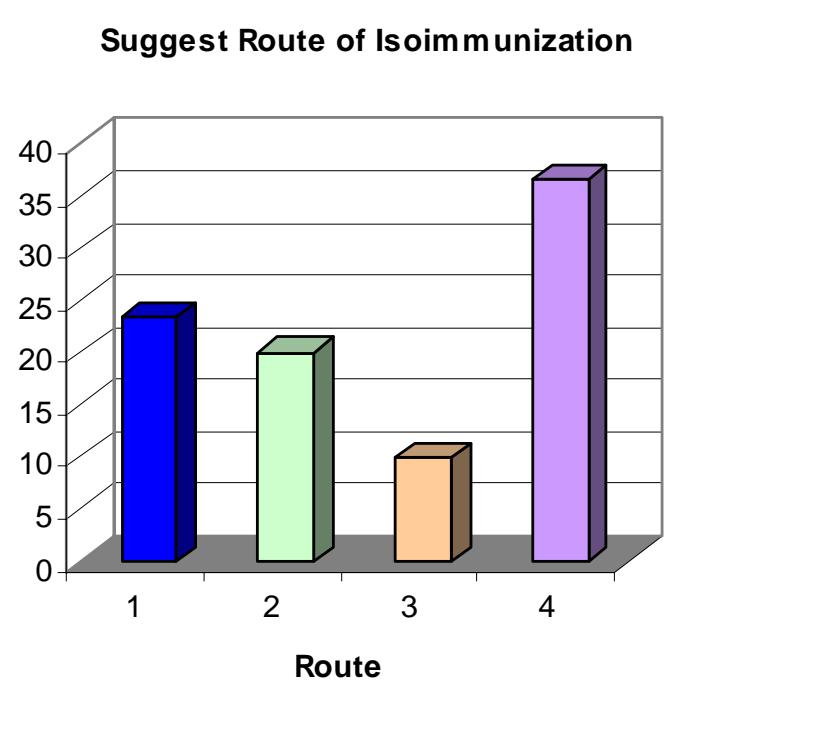


Clinical Impact Analysis

Profile of Patients

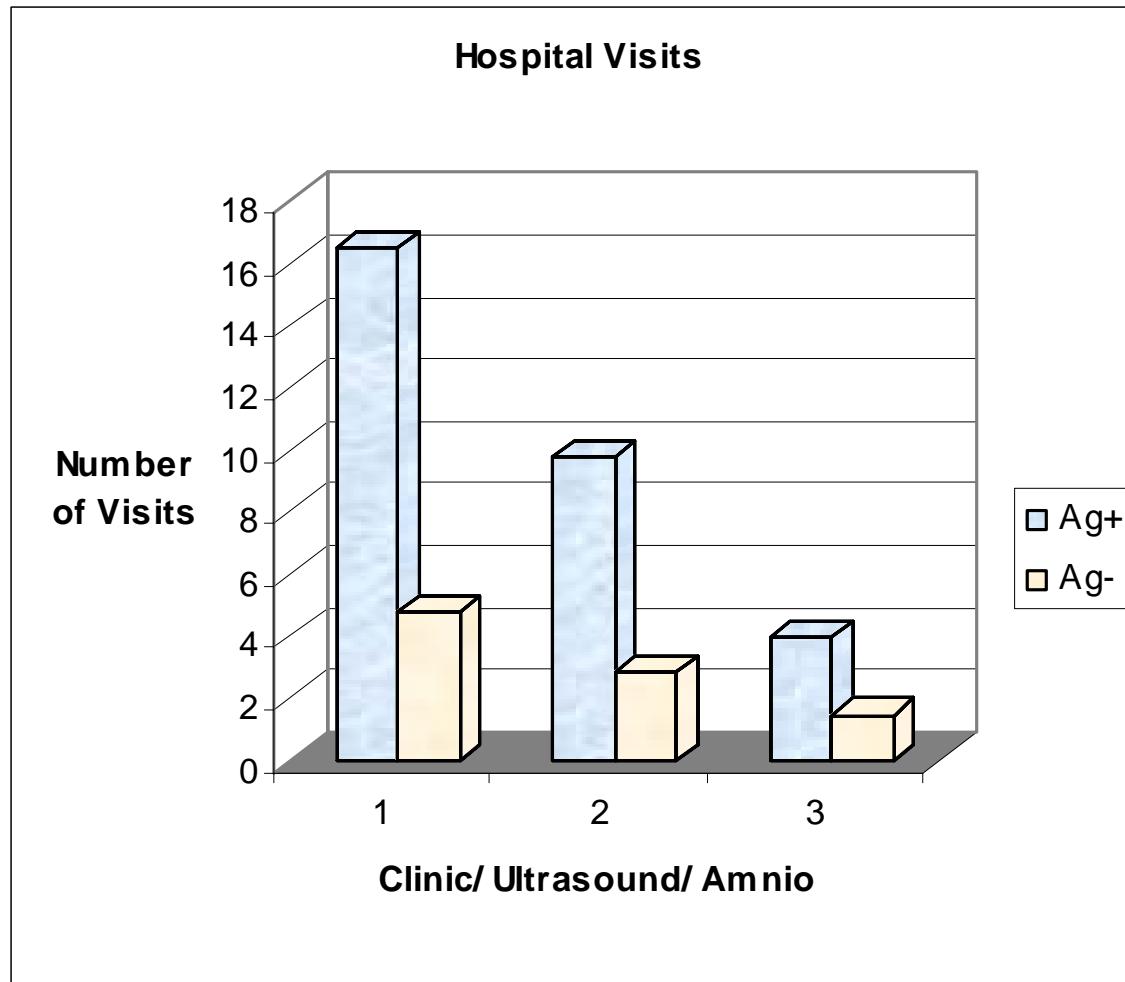


1 = mild (phototherapy)
2 = moderate (phototherapy + ExT)
3 = severe (IUT + ExT + phototherapy)
4 = extreme (severe + fetal demise)

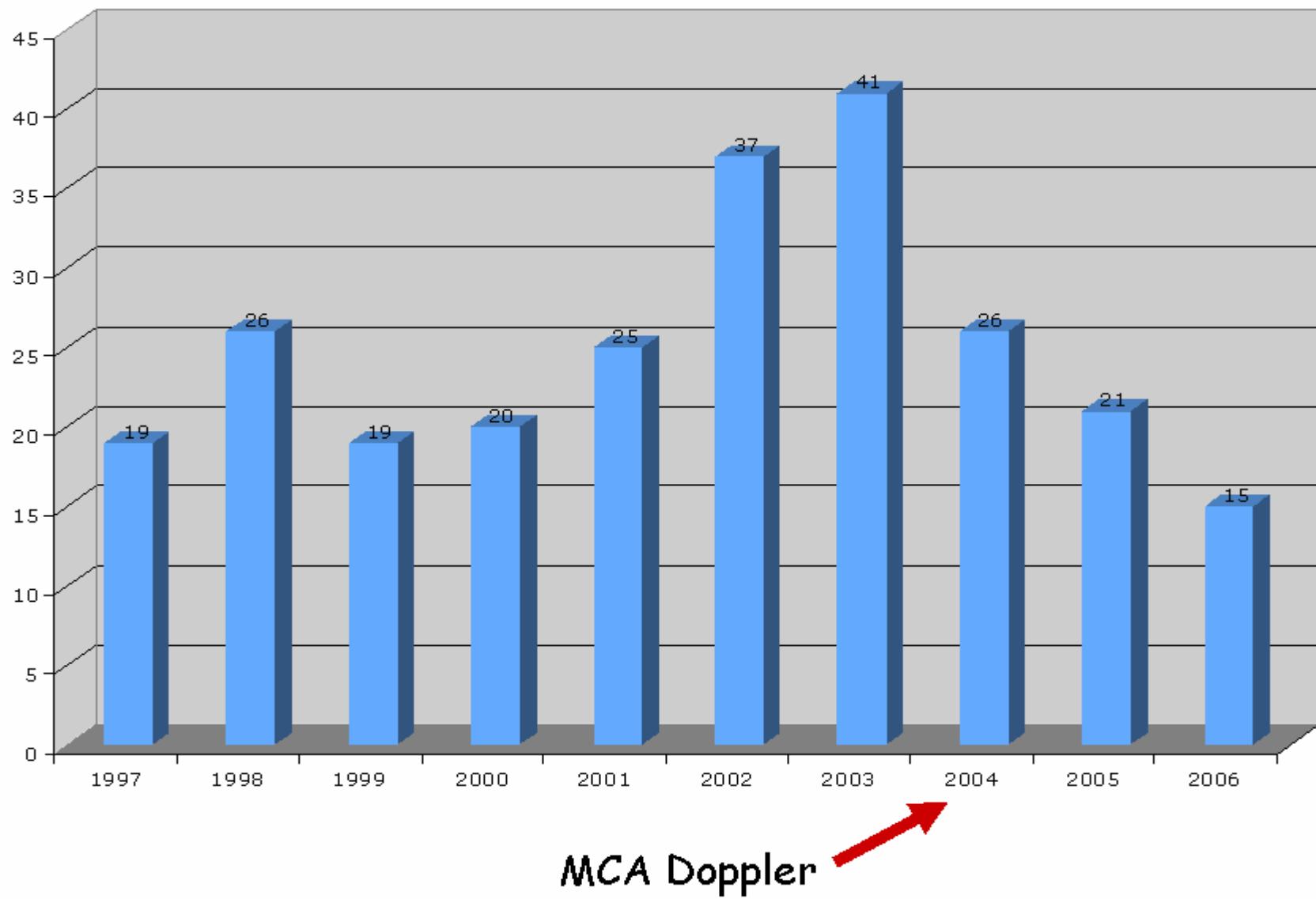


1 = Abortion
2 = Transfusion
3 = Missed Rh immune globulin
4 = Unknown

Impact of Predicting Risk



Number of Requests per Year



Fetal DNA in Maternal Plasma

Fetal Typing Summary

Group (yr)	N	Gestation	Accuracy (%)	Controls
Lo ('98)	57	7-41	96	<i>HBB</i>
Finning ('02)	137	9-34	100	none (rpt)
Turner ('03)	31	<20	90	<i>ACTB</i>
Randen ('04)	114	6-38	92	<i>SRY</i>
Rouillac ('04)	851	Σ/n 15.2	99	<i>SRY</i> + (rpt)
Gautier ('05)	283	8-35	100	none (rpt)
Finning ('04)	359	>16	97	<i>SRY</i> + ins/del
van der Schoot	1257	?	>99	<i>SRY</i> + ins/del
Zhou ('05)	98	15-42	94	<i>SRY</i> + ins/del

40% reduction in invasive testing

Closing Remarks

Early 1990s

- To identify fetus at risk for HDFN: D, C, E, c, K, S, Fy
- To determine *RHD* zygosity: paternal inheritance
- To ...
- Fetal blood group genotyping is a reliable predictive genetic test
- Rhesus box and *RHD* gene dosage have high specificity
 - Require additional genetic information
- Fetal blood group genotyping has an impact on the management of HDFN
- Fetal DNA in maternal plasma will replace fetal *RHD* typing
- Role of Fetal DNA in maternal plasma in:
 - Post-partum & antenatal prophylaxis